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Response Of Groundnut Varieties To Groundnut Ringspot Virus (Grsv) In Western Kenya

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Abstract: *Groundnuts (Arachis hypogea) is an annual legume crop grown by small holder farmers in Kenya for its economic and nutritive value. However, its yields have declined upto 680 kg ha^{-1} than its genetic potential of 1690 kg ha^{-1} attributed to pests and diseases. Viruses are among disease causing factors for yield reduction globally. Orthotospovirus include; Tomato spotted wilt virus (TSWV), Groundnut ringspot virus (GRSV), Impatien necrotic spot virus (INSV), Tomato yellow ring virus (TYRV) among others. Groundnut ringspot virus (GRSV) has been reported in South Africa, Ghana, Brazil, USA, Argentina and Canada infecting groundnuts, soy beans tomatoes, among others. GRSV Symptoms have been noted on groundnuts and other plants in Kenya but no report has been documented on occurrence of the virus and groundnuts varieties resistant to GRSV. The objective of this study was; to determine response of groundnut varieties to GRSV in western Kenya. Health tested seeds of ICGV-9991, Red Valencia, Homabay, ICGV-90704, ICGV-12991, CG7, ICGV-99019, SM99568 and ICGV-99048 groundnut varieties planted in 500ml pots of a mixture of sterilized loam, sand and organic manure at a ratio of 2:1:1 respectively in greenhouse to screen for resistance levels of groundnut varieties to GRSV. Positive isolates for GRSV obtained in a survey from western Kenya, were macerated and mechanically inoculated groundnuts genotypes. symptomatic development, observed and recorded at an interval of 5 days for 8 weeks. Leaf samples collected for serological analysis used polyclonal antisera against GRSV. screened groundnuts showed Homabay being more susceptible with incidence of 31 %, followed by ICGV-9991 with incidence of 28 %. SM99568 variety was tolerant to the virus. Varieties ICGV-90704, ICGV-99048 and ICGV-99019 were resistant to the virus. Some of screened plants for host range were symptomatic for the virus and tested positive for the virus. The study showed that GRSV occurs in surveyed counties of western Kenya, which is a big concern. Introgression of resistant genes into local groundnut varieties to gain resistance to the virus be done with urgency.*

Keywords: Groundnuts, Varieties, inoculum, resistance, Screening, GRSV

INTRODUCTION

1.1 Background of the study

Groundnuts (Arachis hypogaea L) is an oilseed legume crop of global importance grown by both smallholder and large commercial producers, for income (Kipkoech *et al.*,2007) and nutritive value (containing 48% edible oil and 25% crude proteins) (Bajpai *et al*.,2017). World annual production is about 44 million tons (USDA, 2018) with China being the largest producer, followed by India, USA, Nigeria and Indonesia respectively (FAOSTAT, 2015). The crop is grown between 40° North and 40° South (Kumar *et al.*, 2007). Groundnut is the 5th most widely grown crop in Sub-Saharan Africa behind maize, sorghum, millet and cassava (Rockstrom *et al*.,2003). Nigeria produces 30% of Africa's total yields, followed by Senegal and Sudan 8%, Ghana and Chad produce 5% total yield of Africa (Upadhyaya *et al*., 2006; Caliskan *et al*., 2008). In Kenya groundnuts are mainly grown in western Kenya and around Lake Victoria region (Ndisio, 2015). They are roasted or boiled and sold as snack in the streets and for manufacture of peanut butter in factories. Despite of its economic importance, yields of groundnuts in Kenya remains lower ;680kg ha^{-1} against its genetic potential of 1690 Kg ha^{-1} (FAO, 2015), due to lack of high yielding varieties, pests and disease (Bucheyeki *et al*.,2008). Among diseases are viral diseases caused by; Tomato spotted wilt virus (TSWV), Groundnut bud necrosis virus (GBNV), Tobacco streak virus (TSV), Groundnut rosette assistor virus (GRAV), Groundnut rosette virus (GRV), Satellite RNA associated with GRV and/or GRAV, Peanut clump virus (PCV), Peanut stripe virus (PStV), Bean common mosaic virus (BCMV), Peanut mottle virus (PeMoV) and Cucumber mosaic virus (CMV) are of economic importance in groundnut production globally. Groundnut ringspot virus (GRSV) having similar biological symptoms to TSWV on infected host plant. Tomato spotted wilt virus also infects groundnuts and tomatoes, watermelon, pepper and soybean (Webster *et al*.,2011). These viruses (GRSV and TSWV) belong to genera Tospovirus with same transmitting vectors. Groundnut ringspot virus in Africa has been reported in South Africa and Ghana on groundnuts (Pappu *et al*., 2009) and soybean (Pietersen *et al*., 2002). In Ghana, GRSV reported co-infecting groundnuts with groundnut rosette disease (Appiah *et al*., 2016). Although GRSV has been documented

occurring in South Africa but no record indicates its distribution, incidence and severity. In Ghana, reported viral infection rates to 69.5% (Appiah *et al*., 2016). This disease was also noted on cucumber (*Cucumis sativus* L.) in Brazil (Spadotti *et al*., 2014), coriander (*Coriandrum sativum* L.), eggplant, pepper, tomato and tomatillo in Florida, USA (Webster *et al*., 2011). Chlorotic ringspots and leaf mosaic are common symptoms in groundnuts (Appiah *et al*., 2016). In peppers and tomatoes, chlorotic and necrotic spots on leaves, deformed leaves and fruits, necrosis of stems and terminal growing points are observed. Early infected tomato plant fruits with uneven ripening of tomato fruits affecting their quality.

3.3 Screening response of groundnuts and other host plants to GRSV in W. Kenya.

3.3.1 Seed Quality Tests.

Seeds of groundnuts, were randomly picked for healthy test. Selected seeds were prepared according to international rules for seed health (ISTA, 2014). The seeds wiped with cotton wool soaked into 70 % Ethanol, then rinsed with distilled water. Then transferred to petri dish water-soaked paper towels and sprouted. Sprouted seed samples were picked for GRSV serological tests.

3.3.2 Planting of groundnut varieties

Seeds of the selected groundnut varieties, planted in 500 ml pots in Sterile soil medium composed of loam, manure and sand soils in a ratio of 2:1:1 in a greenhouse. Three seeds of each variety planted in a 500 ml pot. Each variety replicated three times in pots. Nine Groundnut varieties arranged in a randomized block design in a greenhouse and inoculated with positive GRSV isolates obtained in a survey in western Kenya. Health control of each variety were laid separately in the greenhouse away from the inoculated varieties to avoid contaminations or transmission due to proximity.

3.3.3 Inoculum preparation and inoculation

Symptomatic leaf sample of groundnuts isolates from the survey, serologically tested positive for GRSV, grounded using a sterilized pestle and mortar, with the aid of dust powdered Carborundum 320 grit. Freshly prepared ice-cold 0.01M Potassium Phosphate buffer (K_2HP0_4 + KH_2P0_4), pH 7.0, containing 0.2% Sodium Sulfite and 0.01M Mercaptoethanol (1: 6 [w/v] tissue: buffer), added to the ground tissue, mixed and transferred to a falcon tube, and allowed to stand for 5 minutes in ice, to settle debris at the bottom of tube. The sap kept on ice, until inoculation completed. The Carborundum dusted on plants under study, acted as an abrasive. The inoculum applied gently on the leaf surfaces, using saturated cotton wool swab and excess carborundum and inoculum washed out on the groundnut leaves by spraying gently with sterilized distilled water. Hands washed with detergent, before proceeding to the next inoculation, to prevent contamination. The inoculated plants observed on weekly basis for any viral symptom development and recorded. This was repeated for 8 consecutive weeks. Leafy samples for each variety collected and tested by DAS-ELISA for GRSV causal agents.

leaves roll inwards and develop a bronze cast followed by dark brown flecks. Fruits show uneven ripening, and raised bumps or ring patterns on the surface (Webster *et al*., 2011). GRSV symptoms on soybean are not described. This virus was reported for the first time on tomatoes in Florida and Brazil (Adkins *et al*.,2010). GRSV induce same symptoms to TSWV transmitted by thrips causing necrotic spots and flecks on tomato stems and leaves, chlorotic ringspot on leaves and fruits, deformation of leaves, necrotic lesions on stems and petioles on tomatoes and bumps on

Popularly grown groundnut varieties in western Kenya (Red Valencia, SM99568, CG7, ICGV-12991, ICGV-9991, ICGV-90704, ICGV-99048, ICGV-99019 and Homa bay, were screened for response to GRSV positive inoculum.

3.3.4 inoculation of groundnut varieties

Positive isolates to GRSV, macerated and grounded using a pestle and mortar as described in section 3.3. Groundnut varieties inoculated by gently rubbing the inoculums on leaves dusted with carborundum apart from healthy controls. After inoculation, excess carborundum on groundnut leaves, gently removed by spraying with water. Groundnut varieties were observed for viral symptomatic development after 3 days in inoculation and thereafter on 5 days' basis for 8 consecutive weeks. Data collected included: number of symptomatic groundnut plants per variety (disease incidence) and disease severity (using 1-4 scale). Leaf samples were collected, DAS- ELISA as described in section 3.3. Varieties that test positive for GRSV and displaying viral symptoms were being, regarded as susceptible. Viral titre in each variety determined by taking the average Spectrophotometric absorbance values (at 405nm) for the positive samples. This was used to grade the resistance levels of different varieties to GRSV.

3.1.1 Disease incidence and severity determination

Viral symptoms were recorded to determine the disease incidence and severity in each variety. Disease incidence for each variety calculated as percentage of plants showing GRSV disease symptoms to the total number of plants observed for each variety screened for resistance levels for GRSV. The average incidence and severity for each variety

screened was use as the actual disease incidence and severity per variety. The degree of disease (GRSV) incidences was assessed and analyzed according to (Nono-Womdim, 1996) as the proportion of diseased plants in an area.

$$\text{Incidence} = \frac{\text{No. of plants infected}}{\text{No. of plants observed}} \times 100$$

The presence and absence of viral disease on groundnuts varieties planted scored using a rating scale basing on (Nono-Womdim, 1996) where low incidence=1-20%, moderate incidence= 21-49% and high incidence=50-100%. Disease severity was scored on a scale of 1-4, as described by Lyerly *et al*. (2002) and Nascimento *et al*. (2006), the scale used to evaluate the severity of symptoms of TSWV and adapted for GRSV since they belong in the same genus and have similar symptoms, where

1: plants without symptoms.
2: for leaves with symptoms of yellowing, mosaic, and/or chlorotic spots,
3: plants with symptoms of yellowing, mosaic or chlorotic spots, and height reduction,
4; for chlorotic plants and stunting symptoms.

These symptoms were used to score for disease severity in groundnuts and leaf samples for each screened variety, picked for serological tests (DAS-ELISA) to GRSV.

3.1.2 Enzymes- Linked Immunosorbent Assay (ELISA)

Detection of GRSV viral titre on leaf samples by serological techniques was based on the ability of the specific antibodies to react in the vitro with their antigens (virus particle), used polyclonal antibodies (IgG) for detection. Microtiter plants (Grainer microloan medium binding) was used and the volume for each reactant, kept at 100µl. between incubations, 3 intensive washing steps each lasting 3 min, carried out by repeated soaking of the plates in washing buffer for 4 min. The following buffers used;

Coating buffer, pH 9.6 (per litre)

1.59 g Sodium carbonate (Na_2CO_3).

2.93 g Sodium bicarbonate ($NaHCO_3$)

0.20 g Sodium oxide (Na_2O)

dissolved in 900 ml H_2O

PBS (pH 7.4) phosphate buffer Saline

8.00 g Sodium chloride (NaCl)

0.20 g monobasic potassium phosphate (KH_2PO_4).

1.15 g Dibasic Sodium phosphate (Na_2HPO_4)

0.20 g potassium chloride (KCl)

0.20 g Sodium oxide (Na_2O)

Was dissolved in 900ml H_2O pH and adjusted from 7.4 to 11 with NaOH

PBS- Tween (PBST)

PBS+0.5 ml Tween 20 per litre

Sample extraction buffer (pH 7.4).

PBST+2% pvp (pvp- is polyvinyl pyrrolidone).

Conjugate buffer

PBST+2% pvp+0.2% egg albumin

Substrate buffer

97 ml diethanolamine

600 ml H_2O

0.20 g Sodium oxide (Na_2O)

Adjusted to pH 9.8 with HCl and make up to 1 litre with H_2O.

3.1.3 Double Antibody sandwich ELISA (DAS ELISA)

Double antibody sandwich ELISA done with no modification as per Clark and Adams (1977). For detection of GRSV in groundnuts leaf samples, microtiters plates were coated with GRSV IgG diluted 1:1000 (v/v) in coating buffer and incubated for 4 hours at 37 ^{0}C. Sample extracts added and incubate at 4 ^{0}C. Extracts from healthy groundnuts varieties and those of infected with known GRSV used as negative and positive controls, respectively. IgG- alkaline phosphate conjugates diluted 1:1000 (v/v) in conjugate buffer added and incubated for 2 h at 37 ^{0}C substrate.

4.3 Screening for resistance of groundnut varieties to GRSV

Nine groundnut varieties screened for resistance to GRSV showed variant symptoms; leaf mosaic, chlorotic leaf spots, necrotic leaf spots, chlorotic ringspots, reduced height and stunted growth with different incidences and severity for each variety were observed. Homabay groundnut variety had the highest disease incidence of 42% with disease severity of 3.55, followed by ICGV-9991 variety with disease incidence of 31 % and disease severity of 3. Groundnut varieties: ICGV-90704, SM99568 and ICGV-99019, displayed no disease symptoms. SM99568 variety with no disease symptom but tested positive

to GRSV. (figure 9)

Fig 9. Showing visual symptoms of screened groundnut varieties for resistance to GRSV in response to groundnut positive inoculum. R) CG7 groundnut variety with Leaf chlorosis, reduced height, stunted growth, S) ICGV-9991 groundnut variety with Leaf mosaic, reduced height and necrotic leaf spot T) Red Valencia variety with Chlorotic leaf spot, leaf chlorotic, leaf mosaic U) SM99568 groundnut variety with no disease symptom. V) ICGV-12991groundnut variety with Leaf chlorosis, leaf mosaic, necrotic leafspot. w) Homabay groundnut variety showing Chlorotic ringspot, stunted growth, leaf mosaic and leaf necrotic spots. These are GRSV symptoms of positive isolates collected from survey for inoculation.

Table 7: Screened groundnuts for resistance to GRSV in western Kenya.

ID	Variety	Group	N	Incidence	Severity	Symptoms	ELISA
WKGV001	ICGV-12991	Bunch	8	16	1.8	Leaf chlorosis, leaf mosaic, necrotic leaf spot.	+
WKGV002	CG7	Runners	8	23	2.8	Leaf chlorosis, reduced height, stunted growth, chlorotic ringspot.	+
WKGV003	ICGV-99019	Bunch	8	0	1	Absence of viral disease symptom	_
WKGV004	ICGV-99048	Bunch	8	0	1	Absence of viral disease symptoms.	_
WKGV006	SM99568	Bunch	8	0	1	Absence of viral disease symptoms.	+
WKGV007	ICGV-9991	Bunch	8	31	3	Leaf mosaic, reduced height, chlorotic leaf spot and necrotic leaf spot.	+
WKGV008	Red Valencia	Bunch	8	26	1.66	Chlorotic leaf spot, leaf chlorotic, leaf mosaic.	+
WKGV009	Homabay	Runners	8	42	3.55	Chlorotic ringspot, stunted growth, leaf mosaic, reduced height and leaf necrotic spots	+
WKGV011	ICGV-90704	Runners	8	0	1	Absence of viral disease symptoms	_

DISCUSSION AND CONCLUSION

Groundnut varieties (Red Valencia, ICGV-12991, CG7, ICGV-9991, Homabay, ICGV-99048, ICGV-99019, ICGV-90704 and SM99568) screened for resistance to GRSV had different response to GRSV inoculum. This implies that groundnut genotypes are of diversity genetic materials which gives them a variation in response to GRSV gene interaction and association (Jone,2014). Red Valencia, ICGV-12991, CG7, ICGV-9991 and Homabay displayed disease symptoms similar to inoculum isolates; leaf mosaic, chlorotic leaf spot, necrotic leaf spot, reduced stem height and stunted growth symptoms. Homabay variety was more vulnerable to GRSV with disease incidence of 42% and severity of 3.55, followed by ICGV-9991 with incidence of 31 % and severity of 3, then Red Valencia had an incidence of 26 % with severity of 1.66. This is an indication of these varieties being susceptible to this virus (GRSV) although due to genetic diversity of these varieties resulted into variation in response to the virus (Rubio *et al*.,2013). Disease symptom development in ICGV-12991 and CG7 became more visible and severity increased with time in growth stages of plant development. This gives an indication that some varieties may be having mechanisms of reducing virulence or resisting viral multiplication which slow down viral establishment. But with time their systems become overwhelmed and the symptoms are expressed (Lima *et al*., 2000). Groundnut SM99568 variety phenotypically displayed no disease symptoms of the virus after mechanical sap inoculation, but serologically tested positive for GRSV. This means that the variety has genes which are tolerant to this virus,thus the crop appears to be health but is a host for the virus. This variety when planted will act as primary alternative host for the virus and thus, thrips may pick the virus from them and transmit to other hosts in close proximity. ICGV-99048, ICGV-99019 and ICGV-90704 varieties, displayed no disease symptom after mechanical sap inoculation and tested negative for the virus. These varieties are resistant to the virus (Kazuhiro *et al*.,2018). This implies that these varieties have genetic mechanism of defending themselves from virus infection by RNA silencing and resistant (R) gene-mediated mechanisms (Giuseppe *et al.*, 2021). Therefore, a Non-conventional method to be used to confer virus resistance by transferring primary virus-derived genes from these varieties into susceptible varieties to improve on their resistance (Reddy,2009).

Conclusion

This study revealed groundnut varieties grown in western Kenya have different levels of resistance to GRSV strains infecting groundnuts in western Kenya. There is need for urgent measures to manage GRSV in western Kenya through planting resistant varieties of groundnuts to GRSV or introgression of resistant genes into other groundnut varieties that are susceptible to the virus (Culbreath et al.,2003) which are more are very productive. Tolerant varieties of groundnuts be discouraged from being planted by farmers as acts as source of inoculum to GRSV that will be transmitted by thrips to health crops increasing disease incidence thus lowering crop production.

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